Effect of Myriocin on Atherosclerosis Induced by High Fat High Cholesterol Diet in Rats

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Abstract

Background: Altered sphingolipid (SpL) levels, particularly ceramide (Cer) and sphingomyelin (SM), are involved in obesity-induced endothelial dysfunction and atherosclerosis (AS).

Aim: This study aimed at examining the role of inhibition of serine palmitoyl transferase (SPT) by myriocin as a therapeutic candidate for atherosclerosis model induced by 12 weeks high fat high cholesterol diet (HFHCD) in rats.

Material and Methods: This study was carried out on 30 male wistar rats of local strain which were divided into 3 equal groups. Gr I: Standard diet (SD) fed rats as a control group. Gr II: High fat high cholesterol diet (HFHCD) fed rats. Gr III: HFHCD fed rats treated with myriocin. At the end of the experimental period (12 weeks), lipid profile (LDL-ch, HDL-ch, TG, TC, ox-LDL and plasma ceramide), oxidative, inflammatory and endothelial markers [reduced glutathione (GSH), C-reactive protein (CRP) and total serum nitrite / nitrate (NOx)] were measured. Correlation was done between ceramide and lipid parameters (HDL-ch, TC and ox-LDL). Histopathological examination of tissue samples from aortae and coronaries was done in all groups.

Results: HFHCD produced significant dyslipidemia, increased CRP, decreased GSH and NOx. Cer levels negatively correlated with HDL-ch and positively correlated with TC and ox-LDL. Atherosclerotic changes were evident by histopathological examination (foam cells and vascular smooth muscle cell proliferation). Myriocin succeeded to significantly reverse biochemical and histological results induced by HFHCD.

Conclusion: Myriocin inhibits development and progression of AS via multiple diverse chemical scinarios including suppression of de novo lipogenesis, enhancing HDL-biogenesis, function and turnover, and improving endothelial dysfunction.

Key Words: Atherosclerosis – High fat diet – Sphingolipids – Ceramide – Myriocin.

Introduction

ATHEROSCLEROSIS (AS) is a systemic, multicentric and multistage disease and is considered the corner stone of vascular diseases that cause mortality namely; stroke, myocardial infarction and peripheral vascular disease [1]. AS is a chronic low grade inflammatory disease that ties hyperlipidemia, inflammatory cytokines and dysregulated cellular proliferation [2]. Sphigolipids (SpL) play an important biological role in cell membrane formation, intracellular signal transduction and plasma lipoprotein metabolism, all of which have a role in atherosclerosis (AS) [3]. Ceramide (Cer), the backbone of SpL, is composed of long chain sphingosine (Sph) base in N-linkage to a variety of acyl groups (14,16 & 24) [4]. There are 3 major pathways for Cer biosynthesis: de novo pathway requires serine palmitoyl transferase (SPT) enzyme for condensation of serine and palmitoyl CoA, sphingomylinase-dependant hydrolysis of sphingomylin (SM) and the salvage pathway by re-acylation of sphingosine base by ceramide synthase enzyme as shown in Fig. (1) [5]. SPT is located on endoplasmic reticulum and it is the rate-limiting enzyme in all SpL biosynthesis [6].

After oxidative modification of low density lipoprotein (LDL), oxidized LDL (ox-LDL) has greater ability of subendothelial retention. SM hydrolysis rate in ox-LDL is 5-6 times more than native LDL, also, Cer levels in ox-LDL particles in atherosclerotic lesions is 10-50 times its level in plasma natural LDL [7]. Moreover, glycolipids [glucosyleceramide (Glucer) and lactosylceramide (Lac Cer)] were also contained in appreciable amounts in atheromatous plaques and absent in intact intima [8]. Myriocin, a SPT inhibitor, inhibits the initial step in SpL biosynthetic pathway which could potentially result in modulation of several downstream SpL family members [9].

This body of work suggests that inhibition of SPT (the rate-limiting enzyme in SpL synthesis) by myriocin may be a feasible therapeutic candidate for atherosclerosis induced by 12 weeks of high fat high cholesterol diet (HFHCD) in rats.
Effect of Myriocin on Atherosclerosis Induced

Material and Methods

All procedures were approved by Ethical Committee of Tanta University. This study was carried out on 30 male wistar rats of local strain obtained from animal house of Faculty of Science, Tanta University. Rats were of same age (20 weeks) weighing 180-220gm. They were housed in separate cages at room temperature, at constant 12/12 hours dark/light cycle with free access to water and experimental diet all over the period of the work (12 weeks) which started in December 2015. Rats were randomized into 3 equal groups (each of 10 rats).

I- Control group (SD): Were fed standard chow diet (SD, 60% carbohydrate, 30% protein and 10% fat).

II- High fat high cholesterol diet group (HFH-CD): Were fed HFHCD (45% fat, containing 20% saturated fat + 0.2% cholesterol, 35% carbohydrates and 20% protein) [3]. (Both diets were obtained from Win Lab Pharmaceutical Co).

III- HFHCD + myriocin group: Were fed HFHCD plus myriocin in a dose of 2.2mg/kg diet, each rat consume about 6gm diet/day. At this amount, the dose of myriocin is about 0.3mg/kg B.W./day (Biomol research laboratories Inc.) [10].

Experimental methods:

At the end of experimental period (12 weeks), animals of all groups were fasted over night and in the morning, rats were decapitated to collect blood in EDTA-coated and uncoated tubes. After centrifugation, plasma and serum samples were kept into clean storage aliquots at –80°C until analysis. Tissue samples from aorta and coronaries were dissected, washed and preserved in formalin until histopathological examination.

Lipid profile parameters: Including low density lipoprotein-cholesterol (LDL- ch) [11], high density lipoprotein cholesterol (HDL-ch) [12], triglycerides (TG) [13] and total cholesterol (TC) [14] were measured by enzymatic colorimetric methods (bio-diagnostic, Egypt). The serum level of ox-LDL was measured by ELISA assay and monoclonal antibody against human ox-LDL (sigma-Aldrich, St. Louis, MO) [15]. Total plasma ceramide extraction and quantification by mass spectrometry according to the method described by [16].

Atherosclerotic markers: including reduced glutathione (GSH) was estimated according to the method described by Sedlak and Lindsay [17]. C-reactive protein (CRP) [18] and total serum nitrite/nitrate (NOx) by modified Greiss reaction [19].

Correlation was done between ceramide levels and both (HDL-ch, TC & ox-LDL).

Histopathological examination was done for aortic and coronary samples for atherosclerotic changes and stained by (Hx & E).

Statistical analysis:

Data were expressed as mean ± SD. Statistical difference involving multiple group comparisons were determined by one was ANOVA, followed by Scheffe (F) test. The least significant difference between mean values at (p≤0.05). Pearson correlation coefficient was done using SPSS computer program version 16.
Results

High fat high cholesterol diet (HFHCD) consumption for 12 weeks produced significant elevation of all lipid profile (LDL-ch, TG, TC, ox-LDL and plasma ceramide) except HDL-ch which showed significant decrease when compared to standard diet (SD) fed group ($p \leq 0.05$). HFHCD group also showed significant decrease in oxidative stress marker (GSH) and endothelial function marker (NOx) and significant increase in inflammatory marker (CRP) in comparison to SD group ($p \leq 0.05$) Tables (1,2).

Coadministration of myriocin along with HFHCD for 12 weeks produced significant improvement in all measured lipid profile and atherosclerotic markers when compared to untreated HFHCD group ($p \leq 0.05$). In addition, the improvement in (HDL-ch, GSH, CRP and NOx) by myriocin were insignificant when compared to SD group ($p > 0.05$).

Plasma ceramide levels showed significant negative correlation with HDL-ch and significant positive correlation with TC and ox-LDL along all groups ($p < 0.001$) as shown in Table (3) and Fig. (2).

Table (1): Mean ± SD and statistical significance of lipid profile parameters among studied groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>LSD No. 10</th>
<th>II-HFHCD No. 10</th>
<th>III-HFHCD± Myriocin No. 10</th>
<th>F-value</th>
<th>Overall p-value</th>
<th>Significance*</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL-ch (mg/dl)</td>
<td>105.40±6.48</td>
<td>176.60±9.00</td>
<td>126.10±7.14</td>
<td>231.31</td>
<td>0.001</td>
<td>a b c</td>
<td></td>
</tr>
<tr>
<td>HDL-ch (mg/dl)</td>
<td>39.00±6.73</td>
<td>24.50±4.60</td>
<td>31.80±7.82</td>
<td>12.36</td>
<td>0.05</td>
<td>a</td>
<td></td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>112.80±8.82</td>
<td>147.50±8.18</td>
<td>124.40±7.12</td>
<td>47.91</td>
<td>0.01</td>
<td>a b c</td>
<td></td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>104.90±9.16</td>
<td>165.20±9.36</td>
<td>122.20±11.08</td>
<td>98.30</td>
<td>0.01</td>
<td>a b c</td>
<td></td>
</tr>
<tr>
<td>Ox-LDL (µg/ml)</td>
<td>6.29±2.42</td>
<td>50.00±6.22</td>
<td>30.90±6.90</td>
<td>156.30</td>
<td>0.001</td>
<td>a b c</td>
<td></td>
</tr>
<tr>
<td>Plasma ceramide (nmol/ml)</td>
<td>9.70±3.34</td>
<td>51.00±7.20</td>
<td>17.60±5.08</td>
<td>162.51</td>
<td>0.01</td>
<td>a b c</td>
<td></td>
</tr>
</tbody>
</table>

a: HFHCD Vs SD group. b: HFHCD+myriocin Vs HFHCD group. c: HFHCD + myriocin Vs SD group

Table (2): Mean ± SD and statistical significance of atherosclerotic markers among studied groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>LSD No. 10</th>
<th>II-HFHCD No. 10</th>
<th>III-HFHCD± Myriocin No. 10</th>
<th>F-value</th>
<th>Overall p-value</th>
<th>Significance*</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH mmol/mg protein</td>
<td>7.59±1.00</td>
<td>5.14±0.81</td>
<td>7.54±1.00</td>
<td>22.21</td>
<td>0.001</td>
<td>a</td>
<td></td>
</tr>
<tr>
<td>CRP mg/dl</td>
<td>4.54±0.91</td>
<td>7.62±0.79</td>
<td>5.21±0.64</td>
<td>42.30</td>
<td>0.001</td>
<td>a</td>
<td></td>
</tr>
<tr>
<td>NOx nmol/ml</td>
<td>27.40±4.86</td>
<td>16.90±5.20</td>
<td>22.60±5.08</td>
<td>10.85</td>
<td>0.05</td>
<td>a</td>
<td></td>
</tr>
</tbody>
</table>

a: HFHCD Vs SD group. b: HFHCD+myriocin Vs HFHCD group. c: HFHCD + myriocin Vs SD group

Table (3): Correlation between plasma ceramide and (HDL-ch, TC and ox-LDL).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Ceramide (nmol/ml)</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL-ch (mg/dl)</td>
<td>−0.528</td>
<td>0.003 *</td>
<td></td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>0.938</td>
<td>0.001 *</td>
<td></td>
</tr>
<tr>
<td>ox-LDL (µg/ml)</td>
<td>0.883</td>
<td>0.001 *</td>
<td></td>
</tr>
</tbody>
</table>

Fig. (2): Correlation between ceramide and (HDL-ch, TC, and ox-LDL).
Histopathological examination revealed:

Fig. (3): Photomicrography of section from aorta of SD group showing intact normal intima with intact mucosal elastic tissue and normal musculature (Hx & E x250).

Fig. (4-A&B): Photomicrography of section from coronary (A) and aorta (B) of HFHCD group showing: (A) Endothelial denudation, destruction of musculo-elastic layer with multiple foci of intimal and subintimal foamy cells, also there is medial degeneration with multiple foci of foamy cells (Hx & E x250). (B) Deeper sections from aorta showing patchy areas of medial degeneration studded with foamy cells and proliferating vascular smooth muscle cells (VSMCs) (Hx & E x250).

Fig. (5): Photomicrography of section from aorta of HFHCD + myriocin group showing apparently normal histological structure (non-denuded endothelium, no gross plaques in intima and no foam cell accumulation (Hx & E x250).

Discussion

It is clear from the results of the present work that HFHCD for 12 weeks produced significant derangement in all lipid profile, oxidative, inflammatory and endothelial parameters and this was confirmed by the atherosclerotic changes in aortae and coronaries of all rats. Choi and Snider, 2015 reported that HFD enhances de novo SpL synthesis and turnover resulting in generation of SM, Cer and sphingosine 1phosphate (S1P) [3]. Meanwhile, HFD-stimulated SpL generation may contribute to dysregulated lipid accumulation, cytokine production and insulin resistance (IR) [20]. SpLs are also involved in obesity induced endothelial dysfunction and atherosclerosis [4]. Loidl et al., 2008 reported that oxidation of phospholipids of modified LDL (ox-LDL) activates intracellular acid sphingomylinase (ASMase) and consequently elevates Cer level. Integration of Cer causes clustering of many small lipid rafts in cell membranes into larger microdomains which provide a signaling platforms for transmembrane signal transduction [21].

At the same time ox-LDL is able to traffic across the endothelial barrier through tanscytosis. Transcytosis of ox-LDL is highly enhanced by the hydrophobic Cer molecules which fuse simultaneously in membrane rafts and facilitate lipid raft-dependant tanscytosis [22]. Exogenous Cer administration was found to increase ox-LDL receptors (Lox-1), caveolae-associated proteins (caveolin-1 and cavain-1) in membrane rafts [23]. High plasma level of ox-LDL is now considered as an indicator of AS and subendothelial retention [24].
In addition, ox-LDL dose dependently increases the activity of lactosylceramide synthase by phosphorylating its serine, threonine and tyrosine residues. Increased lac cer promotes cholesterol accumulation and foam cell formation, inhibits cholesterol efflux via ATP-binding cassette transporter A/apolipoprotein A 1 (ABCA/ApoA-1) pathway, induces monocyte adhesion to endothelial cells and induces vascular smooth muscle cell (VSMC) proliferation via signaling cascade involving ROS generation, p44 MAPK activation, nuclear transcription factor c-Fos and cyclin A [8]. However there is another concept that ceramide may act as an intracellular messenger in cell cycle arrest and apoptosis via caspase-dependant and independent pathway [4].

Ceramide has been postulated as a primary lipid mediator of insulin resistance (IR) by altering several mediators of insulin signaling pathways including translocation of insulin response substrate-1 (IRS-1) and protein phosphatase 2A-mediated dephosphorylation of Akt in PI3K/ Akt/NO pathway [4]. IR stimulates overproduction of remnant particles (TG-rich VLDL and LDL) and alters composition of HDL (become small dense particles prone to degradation) through the action of cholesterol ester transfer protein and hepatic lipase. This is a risk factor for AS [25]. Furthermore, Cer can activate reactive oxygen species (ROS) through activation of NADPH-oxidase, xanthine oxidase and mitochondrial electron transport chain (ETC) specifically at Qi site of complex III [26]. Cer was found to promote to promote IL-6 and consequently CRP through direct proinflammatory effects. At the same time, inflammatory cytokines like TNF-α stimulates Cer production and its signal function (vicious circuit) [27]. Cer accumulation also attributes to endothelial dysfunction through reducing nitric oxide (NO) bioavailability via activation of protein phosphatase 2 A (PP2A) which reduces phosphorylation of endothelial nitric oxide synthase (eNOS), the key step required for NO synthesis [28]. Julie et al., 2014 proved that exogenous Cer causes shift of flow induced vasodilatation mediator from endothelial-derived NO to mitochondrial-derived hydrogen peroxide (H2O2) and use of ceramidase can revert the mechanism of dilatation back to NO [26]. However, Glaros et al., 2008 suggested that Cer levels may have a negligible role in mediating inflammation or cholesterol efflux, and therefore did not influence atherogenicity in mouse models [29].

It is clear from results of the present work that all metabolic derangement, oxidative and inflammatory and endothelial dysfunctional parameters have been significantly reversed by myriocin treatment along with HFHCD. Biochemical improvement was corroborated by apparently normal histopathological findings. Previous studies observed that myriocin treatment decreases all SpLs including SM, Cer, S1P and glycosphingolipids in all tissues including macrophages. It was found that the percentage of reduction of all SpLs by myriocin was more in HFD fed rats than normal chow fed rats [30]. Myriocin also reduces cholesterol absorption through reduction of its transporter proteins on apical membrane of enterocytes like Neimann Pick C1 like1 (NPC1 L1) and ATP-binding cassette A1 (ABC-A1). These changes in small intestine seems mostly to be physiologic rather than morphologic [6]. The significant reduction of TG and TC by myriocin is rather related to reduced lipogenesis via suppression of hepatic sterol regulatory element binding protein IC (SREBP-1c) and not due to increased fatty acid oxidation [31]. However, it was found that no significant change in TG and TC after intraperitoneal myriocin [30].

Myriocin significantly increased HDL-ch through enhanced gene expression of apolipoprotein A1 (Apo A1) and lecithin cholesterol acyl transferase; which are the main proteins involved in HDL biogenesis and maturation [32]. HDL and its essential protein Apo A1 protect against AS through process called "reverse cholesterol transport" (RCT) besides their antioxidant and anti-inflammatory function [33]. Myriocin also increases gene expression of transporter proteins [ABCA1, ABCG5 ABCG8 and scavenger receptor class B 1 (SR-B 1)] involved in macrophage specific reverse cholesterol transfer or cholesterol efflux (macrophages can not limit cholesterol uptake and reduction of foam cell formation depends only on cholesterol efflux pathways to prevent AS) [34]. Myriocin also reduces endothelial transcytosis of ox-LDL via decreased expression of Lox-1, caveolin-1 and cavlin-1 transporter proteins in membrane rafts and this inhibits the lipid raft dependant transcytosis [35].

Notably, myriocin increases sensitization of liver to insulin via increased Akt phosphorylation and decreased Cer levels [36]. Myriocin was found to diminish NF-kB and MAPK activation and inflammatory cytokine production. Myriocin decreases cholesterol sequestration in macrophage plasma membrane leading to less inflammatory response consequently, enhances cholesterol efflux. As an inverse relationship was found between macrophage inflammatory response and cholesterol efflux rate [37]. However, Kasumov et al., 2015 reported the reduction in plasma Cer and athero-
sclerosis by myriocin but without any effect on oxidative stress [4].

Conclusion and Recommendation:

Myriocin inhibits atherosclerosis development and progression via multiple diverse chemical scenarios including suppression of de novo lipogenesis, enhancing HDL biogenesis, function and turnover, and improving endothelial dysfunction. Further trials to be coadministered with statins or ezetimibe are recommended.

References


تأثير الميروسين على تصلب الشرايين المستحدث بالغذاء الغني بالدهون والكوليسترول في الفئران

خلفية البحث: يعتبر تصلب الشرايين هو حجر الأساس في العديد من أمراض الأوعية الدموية التي تؤدي إلى الوفاة مثل السكتة الدماغية والبنك الدماغية وغيرها.

هدف البحث: هو دراسة تأثير عقار الميروسين على تصلب الشرايين المستحدث في الفئران بالغذاء الغني بالدهون والكوليسترول لمدة 12 أسبوعًا.

طريقة البحث: أجري البحث على 30 الفئران الذكور قسمت إلى ثلاث مجموعات متساوية.

المجموعة الأولى: مجموعة ضابطة تناولت الوجبة المعتمدة.

المجموعة الثانية: تناولت الفئران الغني بالدهون والكوليسترول.

المجموعة الثالثة: تناولت الفئران الغني بالدهون والكوليسترول + عقار الميروسين.

في نهاية التجربة تم أخذ عينات الدم وقياس كل من:

- نسبة الدهون (منخفضة الكثافة عالية الكثافة، الكوليسترول الكلي، الدهون الثلاثية، الدهون المنخفضة الكثافة ومكاسيد الريتاميد).
- الجلوديال الكلي، البروتين المنخفض وراثي/نيترات.

تم عمل دراسة مستويةً للياقة العضوية في الناتجية لكل المجموعات.

النتائج: تناول الفئران الغني بالدهون والكوليسترول في إحداث زيادة ذات دالة إحصائية في الدهون كلها عند الدهون عالية الكثافة انخفضت انخفاضًا ذا دالة إحصائية كما زاد البروتين المنخفض ونقص كلا من الجلوديال الكلي بصورة ذات دالة إحصائية. كما وجد ارتباط طردي بين السيراميد ونقص الدهون بالكوليسترول والدهون المنخفضة الكثافة ومنخفضة الكثافة وارتفاع عكس بين السيراميد والدهون عالية الكثافة في كل المجموعات. كما وجدت تأثيرات الخلايا الدموية وبيانات في خلايا العضلات في جدران الأوعية الدموية بالدراسة.

الاستنتاج: عقار الميروسين له تأثير فعال في منع حدوث تطور تصلب الشرايين عن طريق خفض الدهون، تقليل الالتهاب وتحسين وظيفة النسيج الطالبي المغلف للأوعية الدموية، يؤدي ذلك إلى تحسين حالة دراسة إمكانية الجمع بينه وبين الأدوية الأخرى لتصبح الشرايين مثل الاستنادات والإريثيبر.